Amino Acids (2010) 38:133–143
DOI 10.1007/s00726-008-0221-8

ORIGINAL ARTICLE

A preparation of *N*-Fmoc-*N*-methyl-α-amino acids and *N*-nosyl-*N*-methyl-α-amino acids

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Received: 14 November 2008 / Accepted: 19 November 2008 / Published online: 3 December 2008

Abstract A convenient route for the synthesis of lipophilic *N*-Fmoc-*N*-methyl-α-amino acids and *N*-nosyl-*N*-methyl-α-amino acids, interesting building blocks to be used for the preparation of *N*-methylated peptides, is presented. Both nosyl- and Fmoc-protected monomers are accessible, so these compounds can be used in solution as well as in solid phase peptide synthesis. The methodology is based on the use of benzhydryl group to protect temporarily the carboxyl function of *N*-nosyl-α-amino acids and on the subsequent methylation of the *N*-nosyl-α-amino acid benzhydryl esters with diazomethane. The benzhydryl esters offer several beneficial features such as simple preparation, stability to methylation and selective deprotection under mild conditions. The overall procedure is highly efficient in that the adopted conditions keep the chiral integrity of amino acid precursors and the process does not require chromatographic purification of the methylated products.

Keywords *N*-Fmoc-*N*-methyl-α-amino acid · *N*-Methyl-*N*-nosyl-α-amino acid · Benzhydryl esters · Diazomethane · Diphenyldiazomethane · *N*-Methylated dipeptides

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Introduction

N-Methylation of short peptide sequences protected on the carboxyl function as methyl esters was performed by a successful procedure that involves the protection of the terminal amino function with the *p*-nitrobenzenesulfonyl group (nosyl group) and the subsequent *N*-methylation of the resulting peptides with an ethereal solution of diazomethane (Di Gioia et al. 2003, 2005). The methylation of *N*-nosyl-protected amino acids and peptides with diazomethane affords the expected methylation of the sulfonamide nitrogen atom after the formation of the methyl ester at the carboxyl function. In peptide synthesis, the methyl ester cleavage is normally performed with difficulty and could cause racemization of the amino acid chiral centers, especially in the case of *N*-methylated amino acids (Schröder and Lübke 1965; McDermott and Benoiton 1973; Cheung and Benoiton 1977). The availability of *N*-nosyl- and *N*-Fmoc-protected *N*-methylated amino acids represents certainly a significant advancement for the incorporation of *N*-methylated amino acids into peptide chains according to the nosyl- and Fmoc-chemistry procedures both in solution and solid phase (Miller and Scanlan 1997, 1998; Gilon et al. 2002; Biron and Kessler 2005; Biron et al. 2006; Di Gioia et al. 2007).

Materials and methods

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 300 and 75 MHz, respectively, with $CDCl_3$ or DMSO-d_6 as solvent. GC/MS analyses were carried out on a HP-5MS (30 m × 0.25 mm,

PhMesiloxane 5%) capillary column. The mass detector was operated in the electron impact ionization mode (EIMS) with an electron energy of 70 eV. Reaction mixtures were monitored by TLC using silica gel 60-F_{254} precoated glass plates. Solvents were purified and dried by standard procedures and distilled prior to use. When required, the reactions were carried out under an inert atmosphere (N_2). The dichloromethane solution of diazomethane was prepared from *N*-methyl-*N*-nitrosourea following a classical procedure (Arndt 1943). The concentration of the diazomethane solution (0.66 M) was obtained by a backtitration performed with a standard benzoic acid solution. All the compounds synthesized using diazoalkanes are obtained pure enough to be directly characterized by instrumental techniques without need for recrystallization.

Caution. Diazomethane is highly toxic. Hence, this reagent must be handled carefully (Arndt 1943). Dichloromethane solutions of diazomethane are stable for long periods if stored on KOH pellets at −20°C.

Preparation of diphenyldiazomethane (**2**)

Benzophenone hydrazone (1.05 mmol) in dry dichloromethane (10 mL) was treated with Magtrieve™ (15 mmol). The mixture was stirred at room temperature and immediately took on a purple color characteristic of diphenyldiazomethane. Oxidation was completed within 15 min, as checked by TLC analysis (EtOAc:hexane = 1:5) of the reaction mixture. The final mixture was used without further work-up.

N-Nosyl-α-amino acid benzhydryl esters **3a–f**: general procedure

A solution of *N*-nosyl-α-amino acid **1a–f** (1 mmol) in dichloromethane was added to the purple solution of diphenyldiazomethane, prepared as previously described (1.05 mmol). The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature. TLC analysis (solvent system A: EtOAc:hexane = 1:5; solvent system B: Et_2O:petroleum ether = 1:1) showed complete conversion of the precursors **1a–f** after 40 min. After the reaction, Magtrieve™ was retrieved by filtration through a celite short pad and the solution was concentrated under reduced pressure to afford the corresponding benzhydryl esters **3a–f** in quantitative yields.

*N-Nosyl-L-valine benzhydryl ester (**3a**)*

Yellow solid, Mp 95–97°C. Rf = 0.38 (solvent system A), 0.53 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.98–8.01 (m, 2 H, *o*-NO_2), 7.82–7.92 (m, 2 H, *m*-NO_2), 7.08–7.36 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.62 (s, 1 H, RCO_2CHPh_2), 5.85 (d, J = 9.2 Hz, 1 H, N*H*), 4.00 (m, 1 H, α-C*H*), 2.24 (m, 1 H, $(CH_3)_2CH$), 0.98–1.02 (m, 3 H, $(CH_3)_2CH$), 0.82–0.88 (m, 3 H, $(CH_3)_2CH$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.1, 149.8, 145.3, 138.9, 128.6, 128.4, 128.2, 127.8, 127.5, 126.9, 126.8, 126.8, 126.6, 124.1, 78.4, 61.3, 31.6, 19.2, 17.0 ppm. GC/MS (EI) *m/z* (%) 257 (100), 186 (20), 167 (54), 122 (35). Anal. calcd for $C_{24}H_{24}N_2O_6S$: C, 61.52; H, 5.16; N, 5.98; O, 20.49; S, 6.84. Found: C, 61.49; H, 5.17; N, 5.96.

*N-Nosyl-D-valine benzhydryl ester (**3b**)*

Yellow solid, Mp 96–98°C. Rf = 0.38 (solvent system A), 0.53 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 9.1 Hz, 2 H, *o*-NO_2), 7.81 (d, J = 9.1 Hz, 2 H, *m*-NO_2), 7.18–7.39 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.65 (s, 1 H, RCO_2CHPh_2), 5.88 (d, J = 9.2 Hz, 1 H, N*H*), 4.02 (m, 1 H, α-C*H*), 2.22 (m, 1 H, $(CH_3)_2CH$), 1.02 (m, J = 6.6 Hz, 3 H, $(CH_3)_2CH$), 0.82 (d, J = 6.6 Hz, 3 H, $(CH_3)_2CH$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.1, 149.8, 145.3, 138.9, 128.6, 128.4, 128.2, 127.8, 127.5, 126.9, 126.8, 126.6, 126.8, 124.1, 78.4, 61.3, 31.6, 19.2, 17.0 ppm. GC/MS (EI) *m/z* (%) 257 (100), 186 (20), 167 (54), 122 (35). Anal. calcd for $C_{24}H_{24}N_2O_6S$: C, 61.52; H, 5.16; N, 5.98; O, 20.49; S, 6.84. Found: C, 61.45; H, 5.15; N, 5.95.

*N-Nosyl-L-leucine benzhydryl ester (**3c**)*

Yellow solid, Mp 95–97°C. Rf = 0.37 (solvent system A), 0.32 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 9.2 Hz, 2 H, *o*-NO_2), 7.82 (d, J = 9.2 Hz, 2 H, *m*-NO_2), 7.12–7.38 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.58 (s, 1 H, RCO_2CHPh_2), 5.52 (d, J = 9.3 Hz, 1 H, N*H*), 4.15 (m, 1 H, α-C*H*), 1.80 (m, 1 H, $(CH_3)_2CHCH_2$), 1.52–1.60 (m, 2 H, $(CH_3)_2CHCH_2$), 0.82–1.03 (m, 6 H, $(CH_3)_2CHCH_2$) ppm. GC/MS (EI) *m/z* (%) 271 (60), 215 (22), 186 (28), 167 (100), 122 (21). Anal. calcd for $C_{25}H_{26}N_2O_6S$: C, 62.23; H, 5.43; N, 5.81; O, 19.89; S, 6.64. Found: C, 62.33; H, 5.41; N, 5.79.

*N-Nosyl-S-benzyl-L-cysteine benzhydryl ester (**3d**)*

Yellow solid, Mp 91–93°C. Rf = 0.53 (solvent system A), 0.42 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 9.1 Hz, 2 H, *o*-NO_2), 7.88 (d, J = 9.1 Hz, 2 H, *m*-NO_2), 7.08–7.42 (m, 15 H, $RCO_2CH(C_6H_5)_2$ and $SCH_2C_6H_5$), 6.70 (s, J = 9.3 Hz, 1 H, RCO_2CHPh_2), 6.06 (d, J = 9.1 Hz, 1 H, N*H*), 4.35 (m, 1 H, α-C*H*), 3.60–3.70 (m, 2 H, SCH_2Ph), 2.81–2.88 (m, 2 H, CH_2SBzl) ppm. Anal. calcd for $C_{29}H_{26}N_2O_6S_2$: C, 61.90; H, 4.66; N, 4.98; O, 17.06; S, 11.40. Found: C, 62.03; H, 4.65; N, 4.97.

*N-Nosyl-L-isoleucine benzhydryl ester (**3e**)*

Yellow solid, Mp 92–94°C. Rf = 0.46 (solvent system A), 0.51 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.85 (d, J = 9.2 Hz, 2 H, *o*-NO_2), 7.75 (d, J = 9.2 Hz, 2 H, *m*-NO_2), 7.21–7.40 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.58 (s, 1 H, RCO_2CHPh_2), 5.60 (s, J = 9.3 Hz, 1 H, N*H*), 3.98 (d, J = 8.3 Hz, 1 H, α-C*H*), 1.90 (m, 1 H, C*H*(CH_3) CH_2CH_3), 1.50 (m, 1 H, –$CH(CH_3)CH_2CH_3$), 1.22 (m, 1 H, $CH(CH_3)CH_2CH_3$), 0.94 (d, J = 4.8 Hz, 3 H, –$CH(CH_3)$ CH_2CH_3), 0.81 (t, J = 7.3 Hz, 3 H, $CH(CH_3)CH_2CH_3$) ppm. GC/MS (EI) *m/z* (%) 271 (60), 186 (28), 167 (100), 122 (21). Anal. calcd for $C_{25}H_{26}N_2O_6S$: C, 62.18; H, 5.43; N, 5.81; O, 19.89; S, 6.64. Found: C, 62.23; H, 5.41; N, 5.82.

*N-Nosyl-L-alanine benzhydryl ester (**3f**)*

Yellow solid, Mp 100–102°C. Rf = 0.26 (solvent system A), 0.34 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 9.2 Hz, 2 H, *o*-NO_2), 7.88 (d, J = 9.2 Hz, 2 H, *m*-NO_2), 7.19–7.38 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.64 (s, 1 H, RCO_2CHPh_2), 5.76 (d, J = 9.1 Hz, 1 H, N*H*), 4.23 (m, 1 H, α-C*H*), 1.48 (d, J = 6.0 Hz, 3 H, CH_3) ppm. GC/MS (EI) *m/z* (%) 229 (44), 186 (18), 167 (100), 122 (15). Anal. calcd for $C_{22}H_{20}N_2O_6S$: C, 59.99; H, 4.58; N, 6.36; O, 21.79; S, 7.28. Found C, 59.99; H, 4.59; N, 6.34.

N-Methyl-*N*-nosyl-α-amino acid benzhydryl esters **4a–f**: general procedure

A 0.66 M solution of diazomethane in dry dichloromethane (8 mmol) was cautiously added dropwise to a magnetically stirred solution of the *N*-nosyl-α-amino acid benzhydryl esters **3a–f** (1 mmol) in dry dichloromethane (10 mL). The resulting mixture was stirred at room temperature. TLC analysis (solvent system A: EtOAc:hexane = 1:5; solvent system B: Et_2O:petroleum ether = 1:1) showed the complete conversion of the precursors **3a–f** after 1.5 h. Evaporation of the solvent under reduced pressure afforded the *N*-methyl-*N*-nosyl-α-amino acid benzhydryl esters **4a–f** in quantitative yields.

*N-Methyl-N-nosyl-L-valine benzhydryl ester (**4a**)*

Yellow solid, Mp 91–93°C. Rf = 0.50 (solvent system A), 0.64 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 8.4 Hz, 2 H, *o*-NO_2), 7.78 (d, J = 8.4 Hz, 2 H, *m*-NO_2), 7.16–7.38 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.62 (s, 1 H, RCO_2CHPh_2), 4.35 (d, J = 10.5 Hz, 1 H, α-C*H*), 2.92 (s, 3 H, NCH_3), 2.18 (m, 1 H, $(CH_3)_2CH$), 1.05 (d, J = 6.3 Hz, 3 H, $(CH_3)_2CH$), 0.88 (d, J = 6.3 Hz, 3 H, $(CH_3)_2CH$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 168.9, 149.8, 145.3, 138.9, 128.8, 128.5, 128.4, 128.3, 128.2, 127.0, 126.9, 124.3, 77.9, 65.2, 30.3, 28.2, 19.3 ppm. GC/MS (EI) *m/z* (%) 271 (100), 186 (20), 167 (62), 122 (35). Anal. calcd for $C_{25}H_{26}N_2O_6S$: C, 62.23; H, 5.43; N, 5.81; O, 19.89; S, 6.64. Found: C, 62.15; H, 5.42; N, 5.79.

*N-Methyl-N-nosyl-D-valine benzhydryl ester (**4b**)*

Yellow solid, Mp 95–97°C. Rf = 0.50 (solvent system A), 0.64 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 8.4 Hz, 2 H, *o*-NO_2), 7.75 (d, J = 8.4 Hz, 2 H, *m*-NO_2), 7.14–7.35 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.67 (s, 1 H, RCO_2CHPh_2), 4.39 (d, J = 10.5 Hz, 1 H, α-C*H*), 2.94 (s, 3 H, NCH_3), 2.21 (m, 1 H, $(CH_3)_2CH$), 1.03 (d, J = 6.3 Hz, 3 H, $(CH_3)_2CH$), 0.85 (d, J = 6.3 Hz, 3 H, $(CH_3)_2CH$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 168.9, 149.8, 145.3, 138.9, 128.8, 128.5, 128.4, 128.3, 128.2, 127.0, 126.9, 124.3, 77.9, 65.2, 30.3, 28.2, 19.3 ppm. GC/MS (EI) *m/z* (%) 271 (100), 186 (20), 167 (62), 122 (35). Anal. calcd for $C_{25}H_{26}N_2O_6S$: C, 62.23; H, 5.43; N, 5.81; O, 19.89; S, 6.64. Found: C, 62.41; H, 5.44; N, 5.79.

*N-Methyl-N-nosyl-L-leucine benzhydryl ester (**4c**)*

Yellow solid, Mp 93–95°C. Rf = 0.57 (solvent system A), 0.58 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 9.0 Hz, 2 H, *o*-NO_2), 7.75 (d, J = 9.0 Hz, 2 H, *m*-NO_2), 7.18–7.38 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.60 (s, 1 H, RCO_2CHPh_2), 4.84 (m, 1 H, α-C*H*), 2.90 (s, 3 H, NCH_3), 1.69–1.78 (m, 3 H, $(CH_3)_2CHCH_2$ and $(CH_3)_2CHCH_2$), 1.03 (d, J = 6.0 Hz, 3 H, $(CH_3)_2CHCH_2$), 0.99 (d, J = 6.0 Hz, 3 H, $(CH_3)_2CHCH_2$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.8, 144.3, 139.1, 130.0, 128.6, 128.5, 126.7, 124.0, 78.0, 57.7, 38.2, 30.0, 24.5, 23.0, 21.0 ppm. GC/MS (EI) *m/z* (%) 285 (100), 186 (22), 167 (42), 122 (15). Anal. calcd for $C_{26}H_{28}N_2O_6S$: C, 62.89; H, 5.68; N, 5.64; O, 19.33; S, 6.46. Found: C, 63.01; H, 5.69; N, 5.63.

*N-Methyl-N-nosyl-S-benzyl-L-cysteine benzhydryl ester (**4d**)*

Amorphous yellow solid. Rf = 0.62 (solvent system A), 0.63 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 9.1 Hz, 2 H, *o*-NO_2), 7.88 (d, J = 9.1 Hz, 2 H, *m*-NO_2), 7.18–7.42 (m, 15 H, $RCO_2CH(C_6H_5)_2$ and $SCH_2C_6H_5$), 6.70 (s, 1 H, RCO_2CHPh_2), 4.90 (m, 1 H, α-C*H*), 3.76–3.82 (m, 2 H, SCH_2Ph), 2.98 (m, 1 H, CH_2SBzl), 2.81 (s, 3 H, NCH_3), 2.65 (m, 1 H, CH_2SBzl) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 168.1, 149.8, 139.1, 138.9, 137.3, 128.5, 128.4, 128.3, 128.0, 126.7, 124.1,

78.6, 58.6, 35.7, 30.2, 30.1 ppm. Anal. calcd for $C_{30}H_{28}N_2O_6S_2$: C, 62.48; H, 4.89; N, 4.86; O, 16.65; S, 11.12. Found: C, 62.22; H, 4.91; N, 4.84.

*N-Methyl-N-nosyl-L-isoleucine benzhydryl ester (**4e**)*

Yellow solid, Mp 100–102°C. Rf = 0.59 (solvent system A), 0.70 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.88 (d, J = 9.0 Hz, 2 H, *o*-NO_2), 7.75 (d, J = 9.0 Hz, 2 H, *m*-NO_2), 7.20–7.42 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.60 (s, 1 H, RCO_2CHPh_2), 4.47 (d, J = 12.3 Hz, 1 H, α-C*H*), 2.94 (s, 3 H, NCH_3), 1.97 (m, 1 H, $CH(CH_3)CH_2CH_3$), 1.62 (m, 1 H, $CH(CH_3)CH_2CH_3$), 1.26 (m, 1 H, $CH(CH_3)CH_2CH_3$), 0.95 (t, J = 7.2 Hz, 3 H, $CH(CH_3)CH_2CH_3$), 0.82 (d, J = 6.9 Hz, 3 H, $CH(CH_3)CH_2CH_3$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.0, 149.7, 144.3, 128.3, 128.2, 127.5, 127.0, 124.0, 77.8, 63.8, 34.2, 30.5, 25.3, 15.3, 10.4 ppm. GC/MS (EI) *m/z* (%) 285 (100), 229 (32), 186 (21), 167 (72). Anal. calcd for $C_{26}H_{28}N_2O_6S$: C, 62.89; H, 5.68; N, 5.64; O, 19.33; S, 6.46. Found: C, 62.96; H, 5.64; N, 5.67.

*N-Methyl-N-nosyl-L-alanine benzhydryl ester (**4f**)*

Yellow solid, Mp 102–105°C. Rf = 0.44 (solvent system A), 0.57 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.02 (d, J = 9.3 Hz, 2 H, *o*-NO_2), 7.80 (d, J = 9.3 Hz, 2 H, *m*-NO_2), 7.10–7.42 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.68 (s, 1 H, RCO_2CHPh_2), 4.93 (m, 1 H, α-C*H*), 2.85 (s, 3 H, NCH_3), 1.52 (d, J = 7.2 Hz, 3 H, CH_3) ppm. GC/MS (EI) *m/z* (%) 243 (100), 186 (26), 167 (64), 122 (20). Anal. calcd for $C_{23}H_{22}N_2O_6S$: C, 60.78; H, 4.88; N, 6.16; O, 21.12; S, 7.06. Found: C, 60.95; H, 4.87; N, 6.17.

Synthesis of *N*-methyl-*N*-nosyl-α-amino acid **5a–f**: general procedure

To a solution of the *N*-methyl-*N*-nosyl-α-amino acid benzhydryl esters **4a–f** (1 mmol) in dichloromethane (2 mL), trifluoroacetic acid (5–7 mL) and toluene (2 mmol) were added. The resulting mixture was stirred at room temperature for 1 h. After evaporation of the solvent under reduced pressure, saturated aqueous Na_2CO_3 was added and the aqueous solution was extracted with dichloromethane (3× 20 mL). Aqueous 2 N HCl was then added and the acidified solution was extracted with EtOAc (3× 20 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford the *N*-methyl-*N*-nosyl-α-amino acid **5a–f** in 94–98% overall yields. Spectroscopic data of **5a–f** matched those obtained for the same compounds as reported elsewhere (Di Gioia et al. 2007).

*N-Methyl-N-nosyl-L-valine (**5a**)*

Yield 94%. Anal. calcd for $C_{12}H_{16}N_2O_6S$: C, 45.56; H, 5.10; N, 8.86; O, 30.35; S, 10.14. Found: C, 45.46; H, 5.11; N, 8.85.

*N-Methyl-N-nosyl-D-valine (**5b**)*

Yield 94%. Anal. calcd for $C_{12}H_{16}N_2O_6S$: C, 45.56; H, 5.10; N, 8.86; O, 30.35; S, 10.14. Found: C, 45.62; H, 5.09; N, 8.87.

*N-Methyl-N-nosyl-L-leucine (**5c**)*

Yield 94%. Anal. calcd for $C_{13}H_{18}N_2O_6S$: C, 47.26; H, 5.49; N, 8.48; O, 29.06; S, 9.71. Found: C, 47.34; H, 5.50; N, 8.46.

*N-Methyl-N-nosyl-S-benzyl-L-cysteine (**5d**)*

Yield 95%. Anal. calcd for $C_{17}H_{18}N_2O_6S_2$: C, 49.74; H, 4.42; N, 6.82; O, 23.39; S, 15.62. Found: C, 49.91; H, 4.41; N, 6.83.

*N-Methyl-N-nosyl-L-isoleucine (**5e**)*

Yield 98%. Anal. calcd for $C_{13}H_{18}N_2O_6S$: C, 47.26; H, 5.49; N, 8.48; O, 29.06; S, 9.71. Found: C, 47.32; H, 5.50; N, 8.47.

*N-Methyl-N-nosyl-L-alanine (**5f**)*

Yield 98%. Anal. calcd for $C_{10}H_{12}N_2O_6S$: C, 41.66; H, 4.20; N, 9.72; O, 33.30; S, 11.12. Found: C, 41.52; H, 4.20; N, 9.73.

Removal of the nosyl group from *N*-methyl-*N*-nosyl-α-amino acid benzhydryl esters **4a–f**: synthesis of **6a–f**—general procedure

To a solution of **4a–f** (1 mmol) in dry acetonitrile (10 mL), mercaptoacetic acid (3 mmol) was added and the mixture was maintained at 50°C. Sodium methoxide (7 mmol) was then gradually added to the solution with a variable amount of methanol to facilitate the sodium methoxide solubilization. The resulting mixture was stirred for 40 min monitoring the conversion of the precursors **4a–f** by TLC (EtOAc:hexane = 1:5) and GC/MS analyses. Aqueous 1 N HCl was then added and the acidified solution (pH 2) was extracted with EtOAc (3× 10 mL). The aqueous phase was basified with saturated aqueous Na_2CO_3 and then extracted

with EtOAc (3× 10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford the *N*-methyl-α-amino acid benzhydryl esters **6a–f** in 90–96% overall yields. Compounds **6a–f** have been characterized only by GC/MS and immediately subjected to the next step.

*N-Methyl-L-valine benzhydryl ester (**6a**)*

Oil (96%). Rf = 0.55. GC/MS (EI) *m/z* (%) 167 (46), 86 (100).

*N-Methyl-D-valine benzhydryl ester (**6b**)*

Oil (96%). Rf = 0.55. GC/MS (EI) *m/z* (%) 167 (46), 86 (100).

*N-Methyl-L-leucine benzhydryl ester (**6c**)*

Oil (95%). Rf = 0.61. GC/MS (EI) *m/z* (%) 167 (42), 100 (100).

*N-Methyl-S-benzyl-L-cysteine benzhydryl ester (**6d**)*

Oil (92%). Rf = 0.46. MS (ESI-TOF) *m/z* calcd for $C_{24}H_{26}NO_2S^+$ 392.1684; found: 392.1697.

*N-Methyl-L-isoleucine benzhydryl ester (**6e**)*

Oil (95%). Rf = 0.63. GC/MS (EI) *m/z* (%) 167 (46), 86 (100).

*N-Methyl-L-alanine benzhydryl ester (**6f**)*

Oil (90%). Rf = 0.50. GC/MS (EI) *m/z* (%) 167 (64), 58 (100).

Synthesis of *N*-nosyl-dipeptides benzhydryl esters **8a–b**: general procedure

The appropriate *N*-methyl-α-amino acid benzhydryl esters **6a–b** (1 mmol) was suspended in an aqueous solution of $NaHCO_3$ (6 mL, pH 8). A solution of *N*-nosyl-D-alanine chloride (1 mmol) in dichloromethane (6 mL) was added gradually and the resulting mixture was stirred at room temperature for 1 h monitoring the conversion of the precursors **6a–b** by GC/MS analysis. After the reaction, the organic layer was separated and the aqueous phase was extracted with three additional portions of dichloromethane (3× 10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford **8a–b** in 89–91% yields.

*N-Nosyl-D-alanyl-N-methyl-L-valine benzhydryl ester (**8a**)*

Amorphous yellow solid (89%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.28 (d, $J = 9.2$ Hz, 2 H, *o*-NO_2), 8.12 (d, $J = 9.3$ Hz, 1 H, N*H*), 8.02 (d, $J = 9.2$ Hz, 2 H, *m*-NO_2), 7.22–7.39 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.88 (s, 1 H, RCO_2CHPh_2), 4.85 (d, $J = 10.8$ Hz, 1 H, C*H*CH$(CH_3)_2$), 4.32 (m, 1 H, C*H*CH_3), 2.75 (s, 3 H, –NCH_3), 2.13 (m, 1 H, C*H*$(CH_3)_2$), 1.2 (d, $J = 6.9$ Hz, 3 H, CH_3), 0.87 (d, $J = 6.9$ Hz, 3 H, CH(C$H_3)_2$), 0.46 (d, $J = 6.9$ Hz, 3 H, CH(C$H_3)_2$) ppm. Anal. calcd for $C_{28}H_{31}N_3O_7S$: C, 60.74; H, 5.64; N, 7.59; O, 20.23; S, 5.79. Found: C, 60.68; H, 5.65; N, 7.58.

*N-Nosyl-D-alanyl-N-methyl-D-valine benzhydryl ester (**8b**)*

Amorphous yellow solid (91%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.14 (d, $J = 9.2$ Hz, 2 H, *o*-NO_2), 8.02 (d, $J = 9.2$ Hz, 2 H, *m*-NO_2), 7.95 (d, $J = 9.3$ Hz, 1 H, N*H*), 7.20–7.42 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.85 (s, 1 H, RCO_2CHPh_2), 4.75 (d, $J = 10.8$ Hz, 1 H, C*H*CH$(CH_3)_2$), 4.30–4.40 (m, 1 H, C*H*CH_3), 2.82 (s, 3 H, NCH_3), 2.20 (m, 1 H, C*H*$(CH_3)_2$), 1.30 (d, $J = 6.9$ Hz, 3 H, –CH_3), 0.87 (d, $J = 6.9$ Hz, 3 H, C*H*$(CH_3)_2$), 0.72 (d, $J = 6.9$ Hz, 3 H, C*H*$(CH_3)_2$) ppm. Anal. calcd for $C_{28}H_{31}N_3O_7S$: C, 60.74; H, 5.64; N, 7.59; O, 20.23; S, 5.79. Found: C, 60.93; H, 5.62; N, 7.61.

Synthesis of *N*-nosyl-dipeptides **9a–b**: general procedure

To a solution of the *N*-nosyl-dipeptide benzhydryl esters **8a–b** in dichloromethane (2 mL), trifluoroacetic acid (5–7 mL) and toluene (2 mmol) were added. The resulting mixture was stirred at room temperature for 1 h. After evaporation of the solvent under reduced pressure, saturated aqueous Na_2CO_3 was added and the aqueous solution was extracted with dichloromethane (3× 20 mL). Aqueous 2 N HCl was then added and the acidified solution was extracted with EtOAc (3× 20 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford **9a–b** in 98% overall yields.

*N-Nosyl-D-alanyl-N-methyl-L-valine (**9a**)*

Yield 89%. Yellow solid. Mp 107–111°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.48 (d, $J = 9.3$ Hz, 1 H), 8.48 (d, $J = 9.0$ Hz, 2 H, *o*-NO_2), 8.05 (d, $J = 9.0$ Hz, 2 H, *m*-NO_2), 4.33–4.42 (m, 1 H, C*H*CH$(CH_3)_2$), 3.88 (m, 1 H, C*H*CH_3), 2.96 (s, 3 H, NCH_3), 2.02 (m, 1 H, C*H*$(CH_3)_2$), 1.14 (d, $J = 7.4$ Hz, 3 H, CH_3), 0.88 (d, $J = 7.0$ Hz, 3 H,

C*H*$(CH_3)_2$), 0.52 (d, $J = 7.0$ Hz, 3 H, C*H*$(CH_3)_2$) ppm. Anal. calcd for $C_{15}H_{21}N_3O_7S$: C, 46.50; H, 5.46; N, 10.85; O, 28.91; S, 8.28. Found: C, 46.48; H, 5.45; N, 10.87.

*N-Nosyl-D-alanyl-N-methyl-D-valine (**9b**)*

Yield 98%. Yellow solid. Mp 105–108°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.53 (d, $J = 9.3$ Hz, 1 H, *o*-NO_2), 8.48 (d, $J = 9.0$ Hz, 2 H, *m*-NO_2), 8.05 (d, $J = 9.0$ Hz, 2 H, N*H*), 4.52 (d, $J = 10.8$ Hz, 1 H, C*H*CH$(CH_3)_2$), 3.88 (m, 1 H, C*H*CH_3), 2.96 (s, 3 H, NC*H*$_3$), 2.02 (m, 1 H, –C*H*$(CH_3)_2$), 1.14 (d, $J = 7.4$ Hz, 3 H, C*H*$_3$), 0.88 (d, $J = 7.0$ Hz, 3 H, CH(C*H*$_3)_2$), 0.52 (d, $J = 7.0$ Hz, 3 H, CH(C*H*$_3)_2$) ppm. Anal. calcd for $C_{15}H_{21}N_3O_7S$: C, 46.50; H, 5.46; N, 10.85; O, 28.91; S, 8.28. Found: C, 46.49; H, 5.43; N, 10.89.

Synthesis of *N*-nosyl-*N*-methyl-dipeptides **10a–b**: general procedure

A 0.66 M solution of diazomethane in dry dichloromethane (8 mmol) was added cautiously dropwise to a suspension of the dipeptides **9a–b** (1 mmol) in dry dichloromethane (10 mL). The resulting mixture was magnetically stirred at room temperature under N_2. Evaporation of the solvent under reduced pressure afforded the *N*-methylated dipeptides **10a–b** in quantitative yields.

*N-Methyl-N-nosyl-D-alanyl-N-methyl-L-valine methyl ester (**10a**)*

Yield 98%. Yellow oil. GC/MS (EI) *m/z* (%) 243 (100), 229 (20), 186 (18), 122 (28), 56 (24). Anal. calcd for $C_{17}H_{25}N_3O_7S$: C, 49.15; H, 6.07; N, 10.11; O, 26.96; S, 7.72. Found: C, 48.98; H, 6.08; N, 10.09.

*N-Methyl-N-nosyl-D-alanyl-N-methyl-D-valine methyl ester (**10b**)*

Yield 98%. Yellow oil. GC/MS (EI) *m/z* (%) 243 (100), 229 (20), 186 (15), 122 (24), 56 (20). Anal. calcd for $C_{17}H_{25}N_3O_7S$: C, 49.15; H, 6.07; N, 10.11; O, 26.96; S, 7.72. Found: C, 49.23; H, 6.06; N, 10.12.

Synthesis of *N*-Fmoc-*N*-methyl-α-amino acid benzhydryl esters **11a–f**: general procedure

The *N*-methyl-α-amino acid benzhydryl esters **6a–f** (1 mmol) were suspended in aqueous $NaHCO_3$ (6 mL, pH 8). A solution of FmocCl (1 mmol) in dichloromethane (6 mL) was added gradually and the resulting mixture was stirred at room temperature for 1 h monitoring the conversion of the precursors **6a–b** by TLC (solvent system A: EtOAc:hexane = 1:5; solvent system B: Et_2O:petroleum ether = 1:1) and GC/MS analyses. After the reaction, the dichloromethane layer was separated and the aqueous phase was extracted with three additional portions of dichloromethane (3× 10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford the *N*-Fmoc-α-amino acid benzhydryl esters **11a–f** in 85–94% overall yields.

*N-Fmoc-N-methyl-L-valine benzhydryl ester (**11a**)*

Yield 92%. Amorphous white solid. Rf = 0.65 (solvent system A), 0.81 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 55:45 mixture of two rotamers A and B δ 7.65–7.90 (m, 8 H), 7.25–7.48 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.99 (s, 1 H, A, RCO_2CHPh_2), 6.94 (s, 1 H, B, RCO_2CHPh_2), 4.76 (d, $J = 10.3$ Hz, 1 H, α-C*H*), 4.50–4.64 (m, 2 H), 4.29 (m, 1 H), 2.87 (s, 3 H, NC*H*$_3$), 2.30 (m, 1 H, A, $(CH_3)_2CH$), 2.18 (m, 1 H, B, $(CH_3)_2CH$), 1.02 (d, $J = 6.5$ Hz, 3 H, A, $(CH_3)_2CH$), 0.96 (d, $J = 6.5$ Hz, 3 H, B, $(CH_3)_2CH$), 0.90 (d, $J = 6.5$ Hz, 3 H, B, $(CH_3)_2CH$), 0.77 (d, $J = 6.5$ Hz, 3 H, A, $(CH_3)_2CH$) ppm. Anal. calcd for $C_{34}H_{33}NO_4$: C, 78.59; H, 6.40; N, 2.70; O, 12.32. Found: C, 78.70; H, 6.41; N, 2.69.

*N-Fmoc-N-methyl-D-valine benzhydryl ester (**11b**)*

Yield 92%. Amorphous white solid. Rf = 0.65 (solvent system A), 0.81 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 55:45 mixture of two rotamers A and B δ 7.62–7.88 (m, 8 H), 7.21–7.42 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 7.00 (s, 1 H, A, RCO_2CHPh_2), 6.94 (s, 1 H, B, RCO_2CHPh_2), 4.80 (d, $J = 10.3$ Hz, 1 H, α-C*H*), 4.52–4.62 (m, 2 H), 4.30 (m, 1 H), 2.88 (s, 3 H, NC*H*$_3$), 2.28 (m, 1 H, A, $(CH_3)_2CH$), 2.12 (m, 1 H, B, $(CH_3)_2CH$), 0.99 (d, $J = 6.5$ Hz, 3 H, A, $(CH_3)_2CH$), 0.95 (d, $J = 6.5$ Hz, 3 H, B, $(CH_3)_2CH$), 0.89 (d, $J = 6.5$ Hz, 3 H, A, $(CH_3)_2CH$), 0.75 (d, $J = 6.5$ Hz, 3 H, B, $(CH_3)_2CH$) ppm. Anal. calcd for $C_{34}H_{33}NO_4$: C, 78.50; H, 6.40; N, 2.70; O, 12.32. Found: C, 78.59; H, 6.41; N, 2.79.

*N-Fmoc-N-methyl-L-leucine benzhydryl ester (**11c**)*

Yield 90%. Amorphous white solid. Rf = 0.67 (solvent system A), 0.75 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 55:45 mixture of two rotamers A and B δ 8.20 (d, $J = 9.3$ Hz, 2 H), 7.82–7.90 (m, 2 H), 7.59–7.68 (m, 4 H), 7.12–7.43 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.76 (s, 1 H, A, RCO_2CHPh_2), 6.61 (s, 1 H, B, RCO_2CHPh_2), 4.78–4.86 (m, 2 H, A + B, α-C*H*), 4.22–4.65 (m, 3 H, Fmoc-C*H* and Fmoc-CH_2), 2.98 (s, 3 H, NC*H*$_3$), 1.71–1.75 (m, 3 H,

$(CH_3)_2CHCH_2$ and $(CH_3)_2CHCH_2$), 0.98–1.20 (m, 6 H, A + B, $(CH_3)_2CHCH_2$) ppm. Anal. calcd for $C_{35}H_{35}NO_4$: C, 78.77; H, 6.61; N, 2.62.

*N-Fmoc-N-methyl-S-benzyl-L-cysteine benzhydryl ester (**11d**)*

Yield 95%. Amorphous yellow solid. Rf = 0.74 (solvent system A), 0.76 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 55:45 mixture of two rotamers A and B δ 7.92–8.02 (m, 4 H, Fmoc-Ar), 7.50–7.60 (m, 2 H, Fmoc-Ar), 7.22–7.45 (m, 17 H, $RCO_2CH(C_6H_5)_2$ and $SCH_2C_6H_5$ and Fmoc-Ar), 6.82 (s, 1 H, A, RCO_2CHPh_2), 6.72 (s, 1 H, B, RCO_2CHPh_2), 4.98 (m, 1 H, A, α-C*H*), 4.85 (m, 1 H, B, α-C*H*), 4.10–4.52 (m, 3 H, Fmoc-C*H* and Fmoc-CH_2), 3.64–3.72 (m, 2 H, CH_2SBzl), 3.08 (m, 1 H, B, CH_2SBzl), 3.02 (m, 1 H, A, CH_2SBzl), 2.86 (s, 3 H, A, NCH_3), 2.81 (s, 3 H, B, NCH_3), 2.45–2.72 (m, 2 H, A + B, CH_2SBzl) ppm. Anal. calcd for $C_{39}H_{35}NO_4S$: C, 76.32; H, 5.75; N, 2.28; O, 10.43; S, 5.22. Found: C, 76.48; H, 5.73; N, 2.29.

*N-Fmoc-N-methyl-L-isoleucine benzhydryl ester (**11e**)*

Yield 94%. Amorphous white solid. Rf = 0.68 (solvent system A), 0.76 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 55:45 mixture of two rotamers A and B δ 8.08 (d, *J* = 9.3 Hz, 2 H), 7.70–7.78 (m, 2 H), 7.52–7.60 (m, 4 H), 7.20–7.48 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.84 (s, 1 H, A, RCO_2CHPh_2), 6.82 (s, 1 H, B, RCO_2CHPh_2), 4.75 (d, *J* = 12.3 Hz, 1 H, α-C*H*), 4.42–4.52 (m, 4 H, A + B, Fmoc-CH_2), 4.15–4.30 (m, 1 H, Fmoc-C*H*), 2.78 (s, 3 H, NCH_3), 1.99 (m, 1 H, A, $CH(CH_3)CH_2CH_3$), 1.92 (m, 1 H, B, $CH(CH_3)CH_2CH_3$), 1.38 (m, 1 H, A $CH(CH_3)CH_2CH_3$), 1.24 (m, 1 H, B, $CH(CH_3)CH_2CH_3$), 1.06 (m, 1 H, $CH(CH_3)CH_2CH_3$), 0.85–0.90 (m, 3 H, $CH(CH_3)CH_2CH_3$), 0.73–0.79 (m, 3 H, $CH(CH_3)CH_2CH_3$) ppm. Anal. calcd for $C_{35}H_{35}NO_4$: C, 78.77; H, 6.61; N, 2.62; O, 11.99. Found: C, 78.57; H, 6.62; N, 2.61.

*N-Fmoc-N-methyl-L-alanine benzhydryl ester (**11f**)*

Yield 88%. Amorphous white solid. Rf = 0.57 (solvent system A), 0.74 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 60:40 mixture of two rotamers A and B δ 7.96 (d, *J* = 9.3 Hz, 2 H), 7.64–7.71 (m, 2 H), 7.12–7.35 (m, 14 H, $RCO_2CH(C_6H_5)_2$ and Fmoc-Ar), 6.90 (s, 1 H, A, RCO_2CHPh_2), 6.88 (s, 1 H, B, RCO_2CHPh_2), 4.95 (m, 1 H, A, α-C*H*), 4.85 (m, 1 H, B, α-C*H*), 4.35–4.48 (m, 3 H, Fmoc-C*H* and Fmoc-CH_2), 2.88 (s, 3 H, B, NCH_3), 2.82 (s, 3 H, A, NCH_3), 1.38–1.41 (m, 3 H, CH_3) ppm. Anal. calcd for $C_{33}H_{31}NO_3$: C, 80.95; H, 6.38; N, 2.86; O, 9.80. Found: C, 81.13; H, 6.37; N, 2.84.

Synthesis of *N*-methyl-*N*-Fmoc-α-amino acid **12a–f**: general procedure

To a solution of the appropriate *N*-methyl-*N*-Fmoc-α-amino acid benzhydryl esters **11a–f** in dichloromethane (2 mL) trifluoroacetic acid (5–7 mL) and toluene (2 mmol) were added. The resulting mixture was stirred at room temperature for 1 h. After evaporation of the solvent under reduced pressure, saturated aqueous Na_2CO_3 was added and the aqueous solution was extracted with dichloromethane (3× 20 mL). Aqueous 2 N HCl was then added and the acidified solution was extracted with EtOAc (3× 20 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford the *N*-methyl-*N*-Fmoc-α-amino acid **12a–f** in 94–98% overall yields. Spectroscopic data of **12a–f** matched those obtained for the same compounds as reported elsewhere (Di Gioia et al. 2007).

*N-Fmoc-N-methyl-L-valine (**12a**)*

Yield 96%. Anal. calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96; O, 18.11. Found: C, 71.40, H, 6.58, N, 3.98.

*N-Fmoc-N-methyl-D-valine (**12b**)*

Yield 96%. Anal. calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96; O, 18.11. Found: C, 71.12; H, 6.57; N, 3.97.

*N-Fmoc-N-methyl-L-leucine (**12c**)*

Yield 98%. Anal. calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81; O, 17.42. Found: C, 71.72; H, 6.87; N, 3.79.

*N-Fmoc-N-methyl-S-benzyl-L-cysteine (**12d**)*

Yield 94%. Anal. calcd for $C_{26}H_{25}NO_4S$: C, 69.78; H, 5.63; N, 3.13; O, 14.30; S, 7.16. Found: C, 69.59; H, 5.64; N, 3.11.

*N-Fmoc-N-methyl-L-isoleucine (**12e**)*

Yield 98%. Anal. calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81; O, 17.42. Found: C, 71.66; H, 6.88; N, 3.80.

*N-Fmoc-N-methyl-L-alanine (**12f**)*

Yield 96%. Anal. calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31; O, 19.67. Found: C, 70.39; H, 5.86; N, 4.30.

Results and discussion

N-Fmoc-*N*-methyl amino acids can be prepared by a well-established procedure (Freidinger et al. 1983) based on the

formation of 5-oxazolidinone intermediates which are successively reduced with triethylsilane and trifluoroacetic acid to the corresponding *N*-methylated derivatives. Other methods already reported consist of the Lewis acid catalyzed reduction of *N*-Fmoc-protected amino acid oxazolidinones (Zhang et al. 2005) and the base mediated alkylation of *N*-sulfonyl- and *N*-carbamoyl-protected amino acids (Aurelio et al. 2004). The aim of this work is the development of a novel and very efficient methodology to prepare *N*-nosyl- and *N*-Fmoc-protected *N*-methylated amino acids using diazomethane as methylating reagent. It is clear that for achieving this goal the α-amino acid carboxyl function needs a preliminary protection with a transient and easily removable blocking group. It seemed to be particularly advantageous to protect the carboxyl moiety as diphenylmethyl (benzhydryl) ester (Aboderin et al. 1965; Stelakatos et al. 1966; Barlos et al. 1987; Wuts and Greene 2007). In fact, the benzhydryl derivatives are ready cleaved by hydrogenolysis (De Bernardo et al. 1985) or acidolysis (Torii et al. 1991; Lowe and Vilaivan 1997) under conditions that usually do not affect the peptide skeleton. Moreover, these compounds can be prepared without difficulty by using diphenyldiazomethane upon neutral conditions (Kwang-Youn and Ji-Yeon 1999). Diphenyldiazomethane, a red crystalline solid, is obtained easily by the oxidation of benzophenone hydrazone with Magtrieve™ under mild and non-toxic reaction conditions (Lee and Donald 1997). Lipophilic α-amino acids were chosen as model systems in order to accurately study the synthetic strategy for the obtainment of the *N*-nosyl- and *N*-Fmoc-protected *N*-methylated amino acids. Subsequently the methodology was also extended to the *N*-nosyl-*S*-benzyl-L-cysteine. The *N*-nosyl-α-amino acid benzhydryl esters **3a–f** were prepared in quantitative yields by treating the corresponding *N*-nosyl-α-amino acids **1a–f** (Di Gioia et al. 2005) with diphenyldiazomethane **2** (Fig. 1; Table 1). The reaction of **3a–f** with diazomethane gave the corresponding *N*-methyl-*N*-nosyl-α-amino acid benzhydryl esters **4a–f** that were recovered in quantitative yields and high purity by evaporation of the solvent under reduced pressure. The reaction of diphenyldiazomethane with the α-amino acid carboxyl function is fast and during the reaction is possible to visually observe a rapid decoloration of the reaction mixture.

The *N*-methylated benzhydryl esters **4a–f** are the key precursors of both *N*-methyl-*N*-nosyl-amino acids and *N*-methyl-*N*-Fmoc-amino acids. The treatment of **4a–f** with 70% trifluoroacetic acid in dichloromethane afforded the corresponding *N*-methylated amino acids **5a–f** in high yields (94–98%) (Fig. 1; Table 1).

An alternative reaction path was developed to obtain from the *N*-methylated benzhydryl esters **4a–f**, the corresponding *N*-methyl-*N*-Fmoc-amino acids. This approach

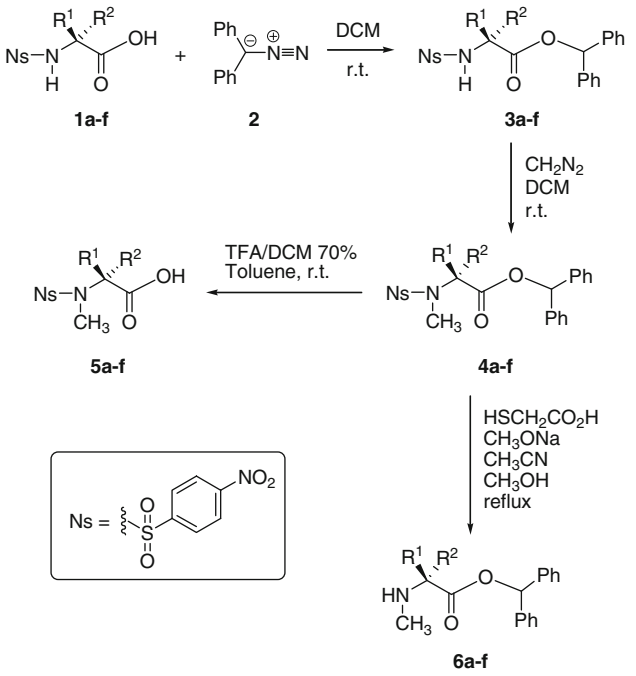

Fig. 1 *N*-Methyl-*N*-nosyl-α-amino acid (**5a–f**) and *N*-methyl-α-amino acid benzhydryl esters (**6a–f**)

Table 1 Results of the synthesis of **5a–f** and **6a–f**

Entry	R^1	R^2	**5** (yield %)[a]	**6** (yield %)[a]
a	$-CH(CH_3)_2$	–H	94	96
b	–H	$-CH(CH_3)_2$	94	96
c	$-CH_2CH(CH_3)_2$	–H	94	95
d	$-CH_2S(Bzl)$	–H	95	92
e	$-CH(CH_3)CH_2CH_3$	–H	98	95
f	$-CH_3$	–H	98	90

[a] Isolated yield

involved the formation, as synthetic intermediates, of the *N*-methyl amino acid benzhydryl esters **6a–f** (Fig. 1). Compounds **6a–f** were isolated and characterized by mass spectrometry methodologies and immediately used for the subsequent transformations in order to avoid side reactions at the amino function.

The stereochemical integrity of the *N*-methylated products **6a–f** was investigated by converting **6a–b** into the corresponding diastereomeric dipeptides **8a–b**. The dipeptides **8a–b** were easily synthesized by coupling **6a–b** with *N*-nosyl-D-alanine chloride **7** under Schotten–Baumann reaction conditions (Di Gioia et al. 2005) (Fig. 2).

The products **8a–b** were recovered in good overall yield (respectively, 89 and 91%) and isolated in high purity grade, without the need for chromatographic purification. ^{1}H-NMR spectra of **8a** and **8b** were different for some

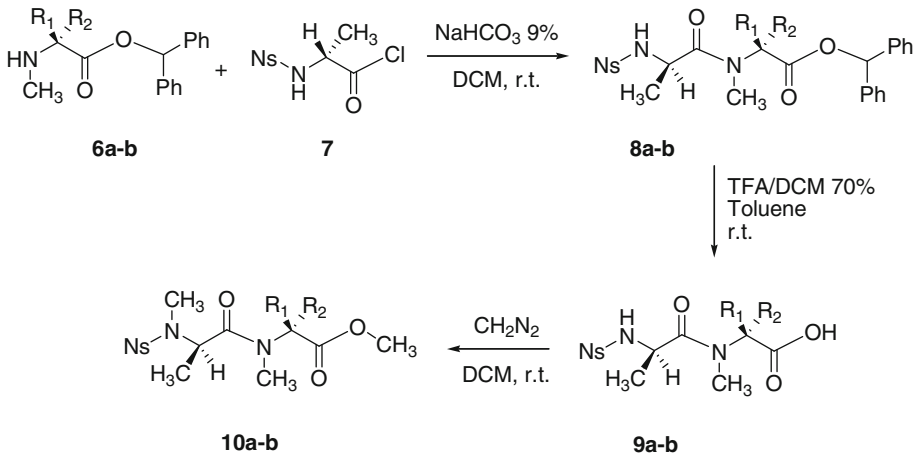

Fig. 2 Synthesis of dipeptides **8a–b** and **10a–b**

signals and showed the presence of a single diastereomer in both samples. The presence of only one diastereoisomeric dipeptide in both crude products distinctly proved that the stereochemistry of the chiral centers is retained throughout the methylation process and the subsequent deprotection of the amino function.

Furthermore, in order to exclude any detectable racemization process the dipeptides **8a** and **8b** were also analyzed by GC/MS, after conversion into the corresponding more volatile methylated dipeptides **10a–b** (Fig. 2). GC/MS analysis performed on an appropriately prepared mixture containing 28 mg of the crude dipeptide **10a** and 70 mg of the crude dipeptide **10b** was compared with those obtained from the single products **10a** and **10b** (Fig. 3).

GC/MS analyses of the single products **10a** and **10b** showed the presence of only one diastereomer in both samples while the two diastereomeric dipeptides **10a** and **10b** appeared readily resolved in the GC/MS analysis of the mixture (Fig. 3). Hence every step of the adopted procedure, the *N*-methylation of the *N*-nosyl-α-amino acid benzhydryl esters up to the deprotection of the amino function does not cause any loss of the chiral integrity of the asymmetric α-carbon atoms of the precursors.

The main goal was to achieve the obtainment of *N*-Fmoc-*N*-methyl-α-amino acids to employ directly as building blocks for the construction of peptide chains based on Fmoc strategy. To this purpose, the synthetic intermediates **6a–f**, obtained from **4a–f** by deprotection of the amino function with the reagent system mercaptoacetic acid/sodium methoxide, were treated with Fmoc-chloride in aqueous 9% $NaHCO_3$ and dichloromethane (Fig. 4).

After complete conversion of the starting reactants, the work-up of the reaction mixture allowed the recovery of the corresponding *N*-methyl-*N*-Fmoc-amino acid benzhydryl esters **11a–f** in high overall yields (88–95%) and with high purity grade (Table 2). The subsequent deprotection of the carboxyl function of **11a–f** by acidolysis with 70% trifluoroacetic acid in dichloromethane afforded the corresponding *N*-methyl-*N*-Fmoc-amino acids **12a–f** in excellent yields (94–98%) (Fig. 4; Table 2). The obtained results demonstrate that the synthesis of *N*-methylated *N*-Fmoc- and *N*-nosyl-protected α-amino acids occurs in a simple way and in high yields.

The method studied here allows for an easy insertion of the Fmoc protecting group at the end of the entire procedure. The inconvenience related to the possible removal of the urethane masking group during the methylation step involved in the base mediated *N*-alkylation methods can be now avoided using the nosyl protecting group which is very stable under acidic and basic environments. Nosyl group represents a valuable improvement for obtaining *N*-Fmoc-*N*-methylated amino acids with respect to the previously appeared works where only *N*-protecting group not sensible to basic conditions were used. Moreover, since diazomethane in our method works under mild and neutral conditions, all the *N*-methylation reactions proceed without racemization. An additional advantage of the method consists in the possibility to work with products that, when protected on the amino function with the nosyl group, are easily analyzed by GC/MS. The fully protected compounds **4a–f** represent the real key intermediates of the developed procedure. They are obtained through the use of a couple of diazoalkanes that react in a rapid, clean and quantitative way at room temperature, and no by-products are observed. Furthermore, **4a–f** can be recovered by a mere evaporation of the reaction solvent without need for chromatography. An additional advantage is the possibility to use highly concentrated diazomethane solutions making the procedure suitable also for gram scale preparation. The *N*-methylated intermediates **4a–f** can also be converted at room temperature into the corresponding *N*-nosyl-α-amino acids in almost quantitative yields. The rapid kinetics of the changing of the amino protecting group and the deprotection of carboxyl function of **4a–f** ensure the recovery of the final *N*-Fmoc-*N*-methylated amino acids in extraordinary

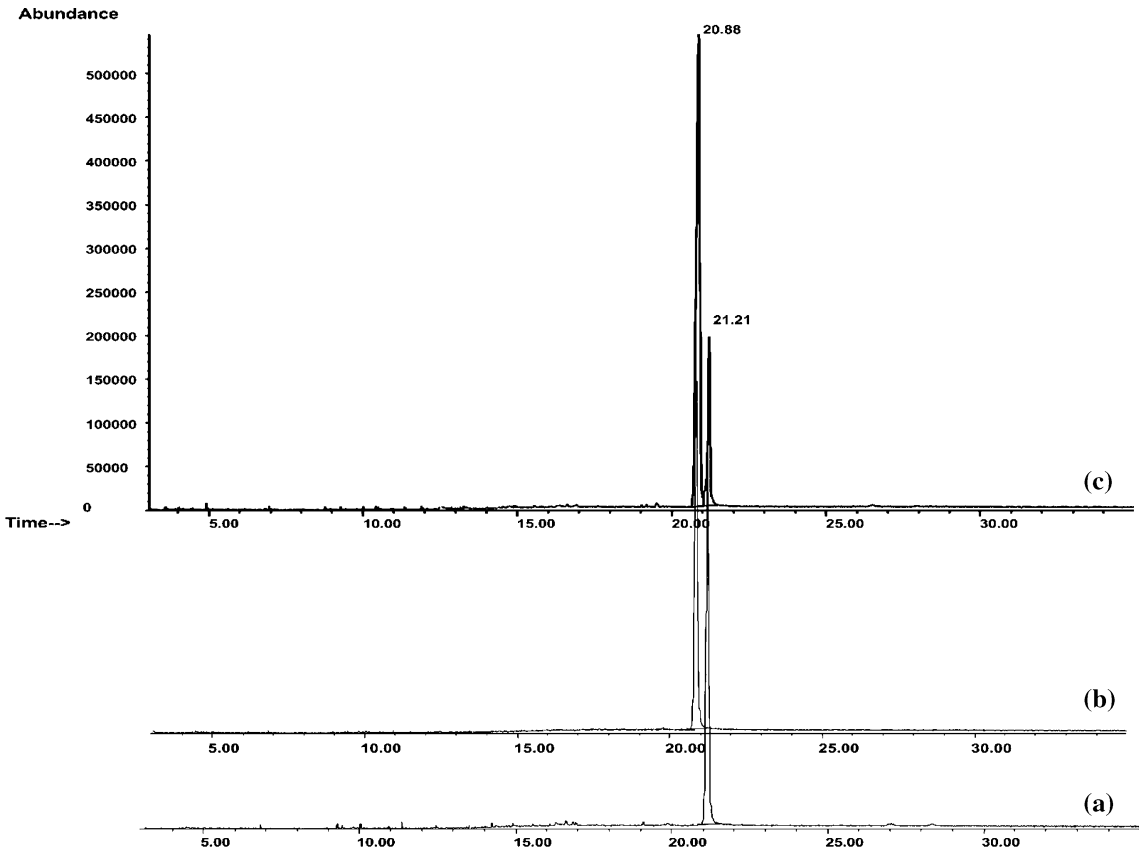

Fig. 3 CG/MS analyses of *N*-nosyl dipeptides: **a** *N*-methyl-*N*-nosyl-D-alanyl-*N*-methyl-L-valine methyl ester (**10a**) (r.t. 21.21 min); **b** *N*-methyl-*N*-nosyl-D-alanyl-*N*-methyl-D-valine methyl ester (**10b**) (r.t. 20.88 min); **c** a mixture of **10a** and **10b**

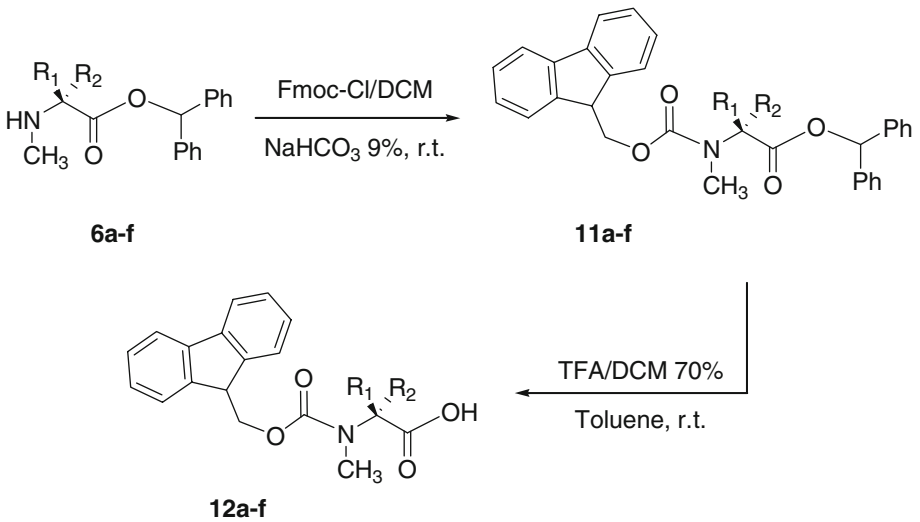

Fig. 4 Synthesis of *N*-Fmoc-*N*-methyl-α-amino acid benzhydryl esters **11a–f** and *N*-Fmoc-*N*-methyl-α-amino acids **12a–f**

high total yields. The methodology here presented allows the straightforward synthesis of the desired *N*-methylated compounds with very short reaction times if compared with the Freidinger's method where a minimum of 22 h is required for the oxazolidinone reduction to afford the corresponding *N*-Fmoc-*N*-methylated derivatives. The process based on the use of diazoalkanes also utilizes reagents less expensive than those employed in the Freidinger's method and offers a smart route for the synthesis of Fmoc-protected *N*-methyl amino acids, avoiding any detectable racemization of the amino acid chiral centers during all the synthetic steps.

Table 2 Results of the synthesis of **11a–f** and **12a–f**

Entry	R^1	R^2	**11** (yield %)[a]	**12** (yield %)[a]
a	$-CH(CH_3)_2$	–H	92	96
b	–H	$-CH(CH_3)_2$	92	96
c	$-CH_2CH(CH_3)_2$	–H	90	98
d	$-CH_2S(Bzl)$	–H	95	94
e	$-CH(CH_3)CH_2CH_3$	–H	94	98
f	$-CH_3$	–H	88	96

[a] Isolated yield

Conclusions

In conclusion we have disclosed herein an experimentally simple and convenient methodology for the preparation of *N*-nosyl- and *N*-Fmoc-*N*-methyl-α-amino acids. The use of diazoalkanes plays a pivotal role in the procedure. The starting materials are *N*-nosyl-α-amino acids protected on the carboxyl function as benzhydryl esters. The benzhydryl group represents an useful carboxyl protecting group by its easy introduction, stability to ongoing methylation reaction, and selective removal under mild conditions. The *N*-methylation with diazomethane of *N*-nosyl-α-amino acid benzhydryl esters provides the key structural precursors of both *N*-nosyl- and *N*-Fmoc-*N*-methylated α-amino acids. In fact, the selective and alternative deprotection of the amino and carboxyl function of *N*-methyl-*N*-nosyl-α-amino acid benzhydryl esters allows to obtain using different reaction paths, the expected *N*-methylated amino acids *N*-nosyl and *N*-Fmoc protected with high yields and purities with no need for chromatography. In light of these argumentations we can conclude that the presented method could be considered as an improvement of the state-of-the-art methodology for the preparation of the title compounds.

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